LIX. THE ESTIMATION OF PYRUVIC ACID.

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Pyruvic acid forms with phenylhydrazine a hydrazone by means of which, as Emil Fischer pointed out, one part of this acid in one thousand parts of water may be detected. The yield of hydrazone however is not quantitative and the attempts made by some authors to estimate pyruvic acid by weighing the phenylhydrazone precipitated have not proved satisfactory. Subsequently nitrophenylhydrazine was used as the precipitating reagent and by this means Neuberg and Karczag [1911] recovered 92 % of pyruvic acid from a 1 % solution, as the nitrophenylhydrazone. In estimating the amount of pyruvic acid in solutions containing 0·1 % and of still lower concentrations this method is however of very little value, since the error introduced by the appreciable solubility of the hydrazone becomes of increasing importance with increasing dilution. An investigation into the action of the tissues on dilute solutions of pyruvic acid which I had undertaken had temporarily to be abandoned since the apparent removal of the acid observed might have been explained by an increase in the amount of hydrazone held in solution, and even when controls of corresponding dilution were used the results were unsatisfactory.

The action of asymmetrical diphenylhydrazine was investigated and was found to present similar difficulties.

If the experimental errors found above were due to the solubility of the hydrazone, the determination of the amount of phenylhydrazine removed from the solution in combination as hydrazone should give satisfactory results.

Estimation of Phenylhydrazine.

Fischer [1878] showed that phenylhydrazine was oxidised by cold dilute Fehling's solution with evolution of nitrogen; benzene and aniline were

formed and cuprous oxide precipitated. Strache and Kitt [1892] estimated the volume of nitrogen liberated and showed that if boiling solutions were used, no aniline was formed and the whole of the nitrogen was liberated in the free state; under these conditions six molecules of cupric oxide were necessary to oxidise two molecules of phenylhydrazine, a mixture of benzene and phenol being obtained. The reaction may be represented as follows:

$$2C_6H_5 . HN . NH_2 + 3O = C_6H_6 + C_6H_5OH + 2N_2 + 2H_2O.$$

The benzene formed during the reaction exerts an appreciable influence on the vapour tension, a difficulty which Strache overcame by saturating the gas both with benzene and with water vapour and introducing the necessary corrections. Strache [1891, 1892] estimated ketones and aldehydes by allowing warm solutions of the carbonyl compound and phenylhydrazine to react and then measuring the excess of phenylhydrazine in the solution by determining the volume of nitrogen evolved when oxidised by boiling Fehling's solution.

The method is not very convenient and it would be preferable to estimate the cuprous oxide formed. By the above method, however, in working with tissue-extracts containing pyruvic acid, any sugar present would react with the boiling Fehling's solution. If, however, the cuprous oxide formed when the excess of phenylhydrazine reacts with Fehling's solution at air temperature be estimated, this difficulty can be obviated.

Experiments were therefore made in order to determine whether the amount of cuprous oxide precipitated by a certain weight of phenylhydrazine was constant.

The Fehling's solution was made up as in Bertrand's method for estimating glucose.

Solution I.	Copper Sulphate crystals 40 grams	per litre.
Solution II.	Rochelle Salt 200 grams Caustic Soda 150 „	per litre.

5 cc. of a solution of phenylhydrazine containing 3·5236 g. in 100 cc. of 50% acetic acid were diluted to 100 cc. with water, and allowed to stand at the ordinary temperature for 30 minutes: in nine experiments, 20 cc. of each of the Fehling's solutions, I and II made up as above, were added to 10 cc. of the diluted phenylhydrazine solution and the mixture allowed to stand at the ordinary temperature for times varying from half an hour to four hours and a half. The cuprous oxide formed was then filtered through a Gooch crucible, dissolved in ferric sulphate solution as in Bertrand's method for the estimation of glucose and the ferrous sulphate produced titrated with deci-normal permanganate solution.

Results.

Time	Cc. N/10 $KMnO_4$ equivalent to Cu_2O formed
30 minutes	6·0
60 ,,	6·05
90 ,,	6·0
150 ,,	6·05
150 ,,	6·0
180 ,,	6·0
210 ,,	6·0
240 ,,	5·95
270 ,,	5·95

The reaction between phenylhydrazine and Fehling's solution appears therefore to reach a definite stage of equilibrium within half an hour at the ordinary temperature, after which no further oxidation proceeds.

In three experiments, the following values were obtained:

1 cc. of N/10 $KMnO_4$ was equivalent to (1) 0·002962 gr. phenylhydrazine,
(2) 0·002962 ,,
(3) 0·002935 ,,

As the mean of these experiments therefore

1 cc. N/10 $KMnO_4$ is equivalent to 0·00295 g. $C_6H_5NH.NH_2$.

It may be mentioned that in filtering the cuprous oxide from the cold Fehling's solution, it is advisable to filter only under a slight difference of pressure, as there is a tendency for the cuprous oxide to pass through the asbestos. If this happens the filtrate should be filtered through a clean Gooch crucible and the two results added together.

Estimation of Pyruvic Acid.

A specimen of Kahlbaum's pyruvic acid was distilled under diminished pressure and the fraction boiling at 77°–78° under a pressure of 15–20 mm. used for the estimation. The method adopted was as follows:

A solution of pyruvic acid was made up containing 1·5228 g. per 100 cc. Quantities of from 2 to 10 cc. of this solution were diluted to about 80 cc., 5 cc. of a solution of phenylhydrazine, approximately 4 %, added, and the mixture made up to 100 cc. and allowed to stand half an hour at the ordinary temperature; 5 cc. of the hydrazine solution were diluted to 100 cc. and allowed to stand for the same time. After half an hour the pyruvic hydrazone which had separated was filtered off and 10 cc. of each filtrate

added to 40 cc. of Fehling's solution. Ten cc. of the control phenylhydrazine solution were similarly treated. The cuprous oxide was estimated as above described.

Thus:

10 cc. phenylhydrazine solution require		6·00 N/10 $KMnO_4$
		6·00 ,, ,,
10 cc. hydrazone filtrate require	...	2·75 ,, ,,
		2·75 ,, ,,

3·25 cc. N/10 $KMnO_4$ are equivalent to $3{\cdot}25 \times 0{\cdot}00295$ g. phenylhydrazine.

$$\underset{108}{C_6H_5HN.NH_2} + \underset{88}{CH_3.CO.COOH} \rightarrow CH_3.C(N_2HC_6H_5).COOH.$$

3·25 cc. N/10 $KMnO_4$ are equivalent to $\frac{88 \times 0{\cdot}00295 \times 3{\cdot}25}{108}$ g. pyruvic acid, and 10 cc. of the diluted pyruvic solution contained 0·00780 g. pyruvic acid, and the original solution contained 1·556 %.

10 cc. of the diluted solution contained by weight 0·007614 g. and the original solution 1·5228 %.

The following table shows some of the results obtained:

Ten cc. of a solution of pyruvic acid contained:

(*a*) By weight	(*b*) By above method of estimation	Error
2·68 milligrams	2·57 milligrams	−0·11 mgr.
3·19	2·15	−1·04
3·57	2·51	−1·06
5·36	5·14	−0·22
6·28	6·57	+0·29
7·14	7·46	+0·32
7·61	7·76	+0·15
8·04	7·71	−0·33
9·42	9·80	+0·38
9·58	9·20	+0·38
10·71	11·22	+0·51
10·71	10·34	−0·37
11·97	12·31	+0·34
12·77	12·67	−0·10
13·40	13·15	−0·25

In carrying out the above estimation it is important that the phenylhydrazine solution shall be freshly made up and if it is at all discoloured that the phenylhydrazine shall be freshly distilled.

Influence of Glucose.

Under the conditions above described the presence of glucose does not appear to interfere with the estimation of pyruvic acid. In one experiment,

5 cc. of a solution of phenylhydrazine acetate, 10 cc. of a solution of pyruvic acid and 10 cc. of a 1 % glucose solution were made up to 100 cc. and the pyruvic acid estimated as above, and compared with a solution similarly made up but from which the glucose was omitted.

Residual hydrazine (without glucose) required 4·80 cc. N/10 $KMnO_4$.
,, ,, (with ,,) ,, 4·85 ,, ,,

The method therefore gives satisfactory results in estimating solutions of concentrations above 0·03 %. Below this concentration probably more accurate results would be obtained by using a more dilute solution of permanganate.

The advantages may be summarised as follows:

(1) It is easily carried out; the whole estimation can be done in little more than an hour.

(2) It gives a greater degree of accuracy than the unsatisfactory method of gravimetric estimation at present in use.

(3) The presence of glucose does not interfere with the estimation.

(4) The method promises to be of general value for the estimation of carbonyl compounds and also for measuring the rate of interaction of these compounds with phenylhydrazine.

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